Reference: *Biol. Bull.*, **156**: 315–327. June, 1979

THE EFFECT OF DIFFERENT ZOOXANTHELLAE ON THE GROWTH OF EXPERIMENTALLY REINFECTED HOSTS

ROBERT A. KINZIE III AND GEORGENE S. CHEE

Zoology Department, University of Hawaii, Honolulu, Hawaii 96822 and Haumāna Biomedical Program, University of Hawaii, Honolulu, Hawaii 96822

The dinoflagellate algae called zooxanthellae are fundamental in the economy of coral reefs. Many aspects of this widespread association have been reviewed (Droop, 1963; Muscatine, Pool and Trench, 1975; and Taylor, 1973a, b, 1974, 1975). Zooxanthellae may enable the symbiotic association to function as a primary producer (Franzisket, 1969, 1970; Johannes, 1974; Porter, 1976); contribute to the general nutrition of the host animal (Muscatine, 1971, 1973; Trench, 1971a, b, c, 1974; Muscatine and Porter, 1977); facilitate calcification in scleractinian corals (Goreau, 1961; Muscatine, 1971; Vandermeulen and Muscatine, 1974; Clausen and Roth, 1975); or function in obtaining or conserving scarce nutrients (D'Elia, 1977, D'Elia and Webb, 1977; Muscatine and D'Elia, 1978). These algae, shown to be *Gymnodinium*-like dinoflagellates by Kawaguti (1944) were first axenically cultured by McLaughlin and Zahl (1959, 1966). They are morphologically very similar across the range of host animals. Early light and electron microscopic studies gave little ground for considering that the symbionts found in different hosts were different (Freudenthal, 1962; Kevin, Hall, McLaughlin and Zahl, 1969). Freudenthal (1962) described the zooxanthellae from the scyphozoan *Cassiopea* sp. as *Symbiodinium microadriaticum*. Taylor (1971a) felt all gymnodinoid zooxanthellae might be but a single pandemic species, which he returned to the genus *Gymnodinium*. More recent workers use the original *Symbiodinium* (Schoenberg, 1976) and both names are found in the current literature. In addition to the nomenclatural differences at the generic level, the question of whether there is a single species of zooxanthella found in almost all marine benthic invertebrates as Taylor suggested (1974), or whether there may be several different zooxanthellae, still remains.

Genetic differences between the zooxanthellae could result from geographical, environmental or host specific factors. McLaughlin and Zahl (1966) suggested that it would be unwise to generalize on the similarity of different zooxanthellae from different habitats. Others (Jokiel and Coles, 1977; Dustan, 1975; and Kinzie, 1970), while giving no evidence from the algae themselves, have interpreted physiological differences between corals in different locations or habitats as suggesting the existence of different algal symbionts. Trench (1971b, c) showed that zooxanthellae from different hosts excrete different amounts of photosynthetic substances under the same conditions *in vitro*. Strains of zooxanthellae isolated from different hosts have been shown to exhibit different isozyme patterns (Schoenberg and Trench, 1976; Schoenberg, 1976). Schoenberg (1976) and Steele (1975) have recently suggested that some strains of zooxanthellae are in fact morphologically different.

While these studies support the idea that there are different kinds of zooxanthellae, the important question (ecologically though perhaps not taxonomically) is whether algal strain differences influence the host/algal association. Advantages may accrue to either the alage or the host and need be neither reciprocal nor symmetrical. Differences in the ability of several strains of zooxanthellae to infect aposymbiotic hosts have been shown by Trench (1971c), Kinzie (1974), and Schoenberg (1976); suggesting that uninfected potential hosts have the ability to discriminate between different algae. Algal growth after reinfection varies. Homologous—"correct"—algae increase in numbers faster than heterologous—"foreign"—strains in these experimental infections (Schoenberg and Trench, 1976; Schoenberg, 1976).

Taken together the studies cited above show that different strains of the zooxanthella *Symbiodinium microadriaticum* exist, and that these strains differ in their ability to infect aposymbiotic coelenterate polyps. Further, the reinfection experiments suggest that those strains that can initiate a symbiotic association differ in their ability to increase in the host's tissues, and that the homologous strain shows the highest growth rate.

The advantages to the host infected with the normal symbiotic algae are well known. A number of workers have shown that in several algal/invertebrate associations the algae contribute to the growth rate or maturation of the host (Muscatine, 1961; Muscatine and Lenhoff, 1965; Karakashian, 1963; Taylor, 1971b; Provasoli, Yamasu and Manton, 1968; Sugiura, 1964). Except for the work of Muscatine on *Anthopleura* and Sugiura on *Mastigias,* these studies have been conducted on systems other than the zooxanthella/coelenterate association.

While anemones of the genus *Aiptasia* are satisfactory test animals for many studies on zooxanthellae/coelenterate interactions, this system suffers from the fact that the polyps generally show neither sexual reproduction in the laboratory nor regularly consistent asexual budding, precluding the use of numerical increase as a measure of growth. Their great contractility and flexibility make linear measurements practically meaningless.

To overcome these problems in assessing the effect of different zooxanthellae on the metabolism of *Aiptasia,* we used the increase in the number of tentacles as a measure of the growth of the host. As will be shown later, the number of tentacles is correlated with the weight of the animal, yet data on tentacle number can be repeatedly obtained from an individual whereas dry weight cannot. This method is sensitive enough to enable us to differentiate between the effects of different zooxanthellae. Preliminary results have shown that this method is an accurate and practical measure of host growth (Kinzie, Araki and Kia, 1977). The experiments discussed here were designed to determine if zooxanthellae isolated from different hosts differ in their ability to contribute to the growth of experimentally reinfected *Aiptasia* polyps.

Materials and Methods

Algae

Homologous algae were isolated from *Aiptasia pulchella* growing in the wet tables supplied with unfiltered running sea water at the Pacific Biomedical Re-

search Center in Honolulu, Hawaii. The tables were outside but shaded by a roof. Algae from several polyps were pooled to initiate the cultures. The other coelenterate host used as a source of zooxanthellae was the scyphozoan *Cassiopea xamachana.* Algae were isolated from three individuals obtained from a depth of about 2 m at the Discovery Bay Marine Laboratory in Jamaica. Two mollusk hosts were used as algal sources. One, *Tridacna maxima,* was obtained from a depth of 3 m near the Mid Pacific Marine Laboratory at Enewetak in the Marshall Islands. Algae were isolated from the mantle tissue of a single individual. Algae from the nudibranch *Melibe pilosa* were isolated from a single individual obtained from a depth of less than 1 m on the Ala Moana reef flat off Kewalo Basin, Oahu, Hawaii. These nudibranchs are found under rocks in the daytime.

The algae were separated from the host tissue by maceration in a Waring Blendor and then in a glass tissue grinder. The macerated material was passed through several layers of cheese cloth to remove large fragments and centrifuged several times to separate algal and animal tissue. All preparations used 0.45 μ Millipore filtered Instant Ocean made with deionized water. Cultures of these isolated zooxanthellae were initiated by innoculating 10-ml tubes containing filter sterilized (0.22 μ) ES enrichment medium (Provasoli, 1966) made with Instant Ocean. Cultures were maintained in an incubator at 25° C with a 12 hr light (28 μEinsteins m^{-2} sec^{-1}: cool white fluorescent lamps)/12 hr dark cycle. Periodic inspections insured that all cultures were unialgal. After several transfers, over a period of at least four months, one-liter flasks containing 600 ml of medium were inoculated with zooxanthellae to obtain sufficient quantities of algae. These cultures were aerated with filtered air and maintained in a light cabinet at ambient temperature, 22–25° C) on a 12 hr light (47 μEinsteins m^{-2} sec^{-1}: cool white fluorescent lamps)/12 hr dark cycle.

Aposymbiotic Aiptasia

Specimens of *Aiptasia pulchella* were obtained from the wet tables at the Pacific Biomedical Research Center, Honolulu. Aposymbiotic polyps were obtained by maintaining them in a darkened 30-liter aquarium in sea water. These bleached animals were fed twice weekly with *Artemia* nauplii hatched in 0.45 μ Millipore filtered Instant Ocean. Two series of experiments were performed with different host material.

Animals used in the first series were kept in the dark for two and a half years. From the time the animals were removed from the dark until the termination of this study, a period of 10 months, none of the *Aiptasia* polyps that were not exposed to algae in the course of the experiments showed any sign of algal infection. Light microscopical study of serial thin sections of these bleached animals showed no trace of any inclusions resembling zooxanthellae.

Because of the small number of these animals available for the experiments, a second series of animals was used. These polyps were from the same source as the animals used in the first series. They were rendered aposymbiotic by maintaining them in the dark for four months. The other conditions were identical. Due to the shorter time without light the bleaching was less successful in this series.

Polyps were maintained in individual 30-ml (first series) or 50-ml (second series) beakers with clear plastic covers. Within each series, polyps were assigned to each of the treatments using a random number table. This was done to remove bias from the initial tentacle numbers and weights. There were no significant differences in tentacle numbers between experimental groups within each of the two series. ($P > 0.5$ and $P > 0.1$ respectively).

Infection

Algae were collected from the flasks and concentrated from the medium by centrifugation. The pellet (about 1–3 ml) was washed several times with 0.22 μ Millipore filtered Instant Ocean. The algae were then mixed with a suspension of ground and filtered *Artemia* nauplii and the mixture was gently centrifuged. After centrifugation, plugs of the pellet were sucked up with a Pasteur pipette and placed on the oral disk of experimental polyps randomly selected from those to be used. In all cases this plug was at least partially swallowed. The *Aiptasia* generally regurgitated some of the ingested mixture within 6 to 12 hr and this material was removed. In each series, controls consisted of uninfected polyps treated in all other ways like the experimental animals. In addition a series of polyps ("normal controls") which had never been bleached were similarly maintained to serve as an estimate of normal growth under the experimental conditions. The initial mean number of tentacles for these normal unbleached controls (12.25 ± 0.71) was between the starting means for the first and second series (9.83 ± 7.93 and 22.37 ± 4.69 respectively).

Maintenance and measurement

The polyps were kept in their individual covered beakers during the course of the experiment. The animals were fed live *Artemia* nauplii hatched in 0.45 μ filtered Instant Ocean once a week. Each polyp received about 40–60 *Artemia* nauplii per feeding. While exact numbers of nauplii were not obtained, efforts were made to normalize the ration. Densities of nauplii were visually adjusted so that each portion was approximately the same. In addition the sequence of feeding was altered each week so that no systematic bias in feeding would occur. It was felt that more precise methods would be fruitless since larger animals had greater catching ability due to longer and more numerous tentacles. Furthermore, the relative amount of food would be smaller for the larger polyps. Also, individual nauplii varied in age and hence yolk content. Finally, in many cases an *Artemia* nauplius would be killed by contact with nematocysts of an *Aiptasia* polyp and fall to the bottom of the beaker where it would remain uneaten. The day after feeding each beaker was cleaned by swabbing the bottom and sides with a disposable cotton applicator to remove detritus and regurgitated or uneaten food, and the 0.45 μ filtered Instant Ocean was changed.

Light was provided by keeping the animals on a shelf next to a window to insure a wide spectral composition. Light values ranged from 15 μEinsteins m^{-2}

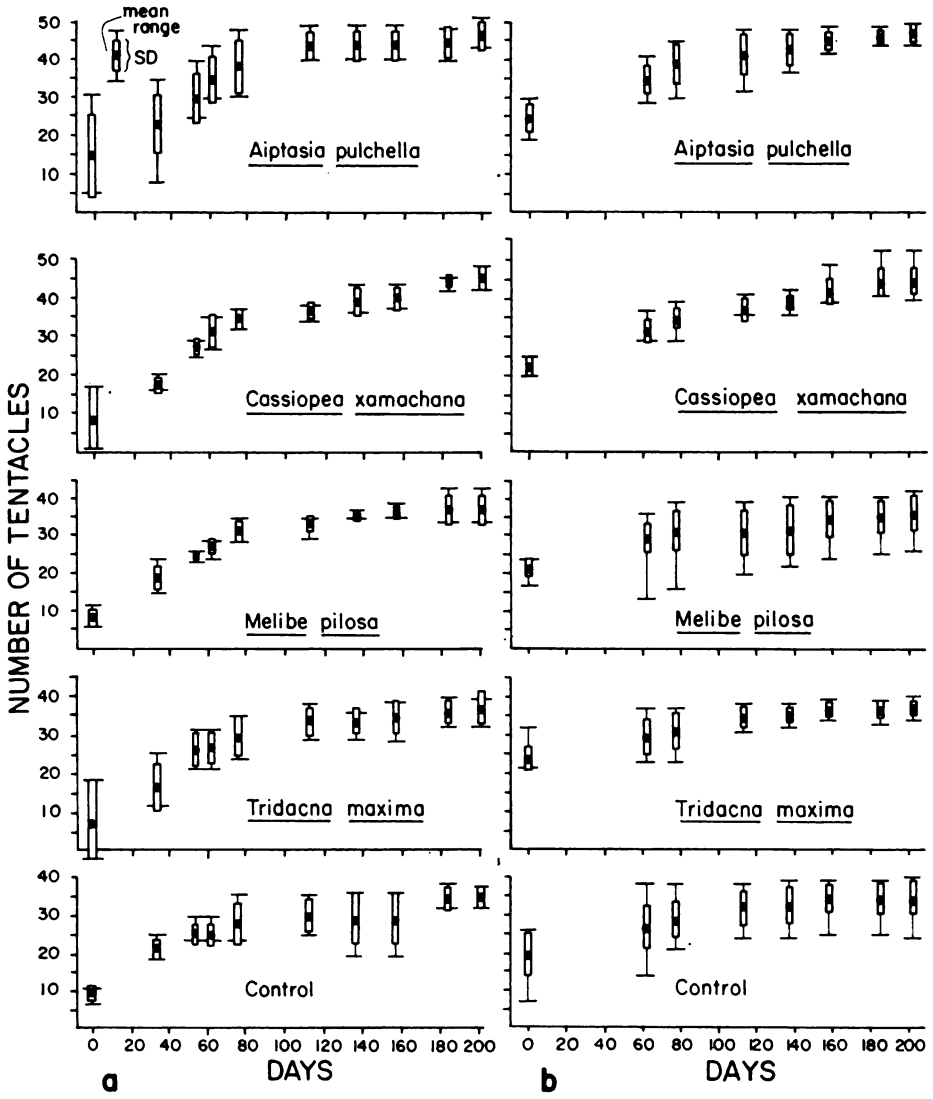

FIGURE 1. The increase in the number of tentacles with time of the first, (A), and second, (B) series of experiments. Means, ranges and one standard deviation on either side of the mean are given. Controls are bleached polyps that were not experimentally infected with zooxanthellae.

sec^{-1} in the morning to 150 μEinsteins m^{-2} sec^{-1} in the afternoon. Temperature varied from 22 to 25° C. At approximately 14-day intervals, growth was measured by counting the number of tentacles of each individual. Experiments were allowed to proceed for over 200 days and observations on state of infection, and general health of the polyps were recorded.

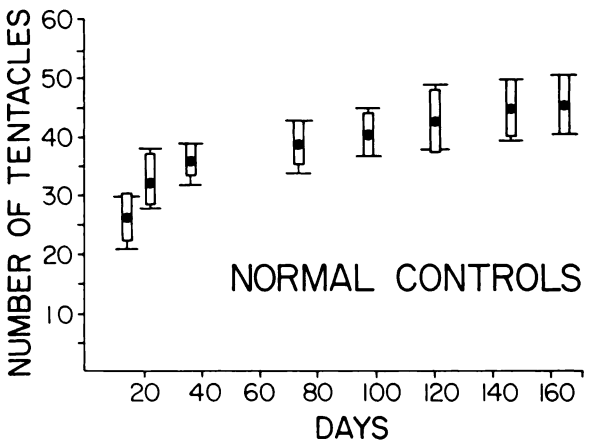

FIGURE 2. The increase in the tentacle numbers of normal control polyps which had never been bleached. These polyps were obtained from the same source as the experimental and control polyps. Since there was no initial infection, the numbering of the days is arbitrarily set at the time when the polyps were isolated.

RESULTS

All of the experimental animals were successfully infected. The polyps became visibly brown within three weeks. In general, the color was first noted in the tentacles. Gradually, the entire body assumed the normal brown coloration.

The results of the tentacle measurements are given in Figures 1 and 2. All the animals in both series, including the normal unbleached controls, exhibited an initial period of increase in tentacle number followed by a more or less asymptotic phase. There are three ways that the growth of the polyps infected with different strains of zooxanthellae could differ: in the final maximum tentacle number; in the rate of increase of tentacles; or in both. To evaluate the first possibility, the

TABLE I

Number of tentacles of Aiptasia *polyps at termination of experiment (202 days) (mean ± one standard deviation).*

Source of algae	Series 1			Series 2		
	$\bar{X}$ s.d.	n	range	$\bar{X}$ s.d.	n	range
Aiptasia	46.25 ± 3.95	4	43–51	47.17 ± 2.79	6	44–50
Cassiopea	45.00 ± 3.00	3	42–48	44.75 ± 3.81	8	40–53
Melibe	37.50 ± 4.04	4	34–43	36.00 ± 4.93	8	26–42
Tridacna	38.75 ± 4.03	4	34–41	36.60 ± 2.07	5	34–39
Uninfected controls	36.00 ± 2.94	4	33–39	35.70 ± 3.06	10	30–40
				(34.00 ± 3.24	5	30–39)*
Normal controls	45.75 ± 4.99	4	41–51			

* Of the ten controls in Series 2, five showed some sign of infection by the last day though only two were visibly infected till that time. The means of the five remaining aposymbiotic are given here; the five showing any traces of infection were not different. ($P > 0.05$)

Analysis of Variance of Final Tentacle Number (Day 202).

Source of variation	Series 1				Series 2			
	df	SS	MS	F	df	SS	MS	F
Among treatments	4	322.2	80.68	5.98**	4	805.2	201.30	15.56**
All infected *vs.* control	1	101.4	101.4	4.21*	1	250.07	250.07	8.55**
Mollusks *vs.* Coelenterates	1	215.4	215.4	16.61***	1	541.5	541.5	35.87***
Aiptasia vs. Cassiopea	1	2.68	2.68	0.21 NS	1	12.6	12.6	0.91 NS
Melibe vs. Tridacna	1	3.13	3.13	0.19 NS	1	0.29	0.29	0.02 NS
within	14	188.5			30	413.0		
Total	18	510.7			34	1,218.2		

results of the different treatments were compared using the number of tentacles on day 202 as the final maximum tentacle number for the polyps. The mean tentacle numbers and statistical comparisons are given in Table I. Because the polyps in each series did not differ in initial tentacle number, the differences in final tentacle number can be attributed to the different treatments. The final tentacle number is markedly greater for polyps infected with zooxanthellae from *Aiptasia* and *Cassiopea*. Neither of these isolates differ significantly in their ability to contribute to the growth of the polyps. Zooxanthellae from *Melibe* and *Tridacna* do not differ from each other, nor do they differ from the uninfected controls, in their ability to support tentacle growth in *Aiptasia*.

The strong agreement between the final tentacle number for polyps infected with each strain in the first and the second series suggests that the possible residual homologous zooxanthellae in the "bleached" polyps in series two did not influence growth. When the bleached controls from the second series that remained visibly uninfected at the end of the experiment were compared with those showing any signs of infection at all, no difference in tentacle number could be found ($t = 2.05$; $P > 0.05$). In addition, the mean number of tentacles for the controls in series one and two was not different ($t = 0.17$; $P > 0.5$), again indicating that the slight degree of infection in the second series did not influence the final tentacle counts. This could be due to the very small number of homologous algae remaining after four months in the dark or to the poor condition of these remaining algae.

To determine if the results were the effects of the infection with different algal isolates or if they were due to the conditions of maintenance, a comparison was made between the experimental animals and the unbleached normal controls which were treated in every other way like the experimental animals. Although the initial tentacle number of these normal controls was between that of the two series, the final tentacle number was not different from the polyps infected with zooxanthellae from *Aiptasia* and *Cassiopea* ($t = 0.01$, $P > 0.95$ first series; $t = 0.02$, $P > 0.95$ second series). This suggests that the experimental infections with these two strains contributed as much to growth as would be expected in a normal polyp kept under the same conditions.

The other aspect of growth that was compared was the rate of increase in number of tentacles. There are two ways that the different treatments could result in the observed differences in final tentacle number. First, the polyps could add tentacles at similar rates but some groups could continue to grow for

TABLE II

Comparison of T_{max} and r for tentacle counts on experimental polyps.

Source of Algae	1st series		2nd series	
	T_{max}	r	T_{max}	r
Aiptasia	48.92 ± 3.36	0.014 ± 0.0037	50.79 ± 3.27	0.010 ± 0.0028
Cassiopea	43.71 ± 1.50	0.020 ± 0.0042	70.13 ± 1.65	0.003 ± 0.0004
Melibe	39.31 ± 1.17	0.015 ± 0.0017	37.86 ± 3.38	0.010 ± 0.0052
Tridacna	39.41 ± 2.21	0.017 ± 0.0036	37.85 ± 1.89	0.012 ± 0.0048
Uninfected control	36.12 ± 1.46	0.016 ± 0.0028	40.45 ± 4.36	0.007 ± 0.0031

Normal control T_{max} = 47.36 ± 3.14 r = 0.015 ± 0.0073

a longer period. Second, the rates of increase could differ, but the period of growth could be the same. The graphs in Figure 1a and b do not permit resolution of these two alternatives. In order to obtain growth rates, the tentacle counts were fitted to a rearranged von Bertalanffy growth equation (von Bertalanffy, 1938). The form used was $T_t = T_{max} - Ge^{rt}$. In this form r is the growth rate, T_{max} is the estimated asymptotic maximum tentacle number, and G is a constant greater than zero. Tentacle counts were fitted to this model using the BMDP P3R non-linear regression procedure.

The results are given in Table II. Two things are apparent from this table. First, with the exception of the polyps infected with zooxanthellae from *Cassiopea,* the T_{max} values are very close to the actual mean tentacle numbers used in the earlier analysis. This is important because the estimation of T_{max} utilizes all the measurement data while the mean final tentacle number discussed earlier uses only the last day's count. The agreement between the number of tentacles on day 202 and the T_{max} value validates the earlier conclusions. The exceptionally high T_{max} value for the polyps infected with zooxanthellae from *Cassiopea* will be discussed below. Second, there are no significant differences between the rates of tentacle increase in any of the experiments or the controls. This suggests that the polyps will all add tentacles at a similar rate. Since those infected with algae from *Aiptasia* and *Cassiopea* and the normal controls achieve higher numbers of tentacles, they must add tentacles over a longer period than uninfected polyps or polyps infected with zooxanthellae from *Melibe* or *Tridacna.*

There are several other measures of the ability of the different strains to enhance the metabolic activities of the experimentally infected *Aiptasia* polyps. A summary of these is given in Table III. The final dry weights of the polyps infected with zooxanthellae from *Aiptasia* and *Cassiopea* are greater than the uninfected controls and the polyps infected with zooxanthellae from *Melibe* or *Tridacna,* (first series $t = 7.46$, $P < 0.001$; second series $t = 4.86$, $P < 0.001$). While initial dry weights of the experimental animals could, of course, not be obtained, the random assignment of polyps within each series to the experimental groups should have equalized the starting weights. The fact that this randomization resulted in no within-series differences in initial tentacle number supports this assumption. In addition, it can be shown that tentacle number is correlated with dry weight. When the final tentacle numbers and the dry weights of polyps in all the

TABLE III

Condition of polyps at end of experiment.

Source of Algae	1st Series			2nd Series		
	Dry wt. mg	No. of pedal lacerates	State of infection	Dry wt. mg	No. of pedal lacerates	State of infection
Aiptasia	4.0 ± 1.0	0.25/ind	All completely infected	3.0 ± 0.6	0.88/ind	All completely infected
Cassiopea	2.6 ± 1.0	1.0/ind	All completely infected	1.7 ± 0.3	1.33/ind	All completely infected
Melibe	0.6 ± 0.2	0.33/ind	1 starting to bleach: others pale	1.5 ± 0.7	0/ind	Six polyps partly bleached two infected
Tridacna	1.2 ± 0.7	0/ind	All completely infected	1.2 ± 0.5	0/ind	All completely infected
Uninfected controls	0.7 ± 1.0	0.40/ind	None infected	1.1 ± 0.5	0/ind	5 completely bleached 1 completely infected 4 partly infected
Normal control	2.3 ± 1.6 mg	2.5/ind	All completely infected.			

experimental groups are compared, correlation coefficients of 0.91 and 0.77 are obtained for the first and second series respectively. A group of *Aiptasia* polyps obtained from the same original source but not kept in beakers also showed a correlation between tentacle number and dry weight ($r = 0.91$). The rate of production of new polyps by pedal laceration is too irregular to allow any unequivocable statements to be made, though it appears that none of the experimental groups produced new polyps as frequently as the normal controls. Polyps infected with zooxanthellae from *Cassiopea* produced the most offspring of the experimental groups in both series.

The final point made by the information in this table is the instability of the infection with zooxanthellae from *Melibe*. In both series, particularly in the second, the polyps infected with algae from *Melibe*, after an initial complete infection, began to lose their algae. This phenomenon was also observed in previous preliminary experiments and seems to be a reproducible phenomenon (cf. Muscatine *et al.*, 1975; Kinzie *et al.*, 1977).

DISCUSSION

While the possibility of the existence of different strains of zooxanthellae has been suggested on indirect grounds for some time, Schoenberg's (1976) work clearly demonstrated that several strains could be differentiated by isozyme electrophoresis, and perhaps more importantly, they would be differentiated by the ability

of the different strains to grow in experimentally infected *Aiptasia* polyps. The results presented here show for the first time that not only do these strains of zooxanthellae exist, but also that they differ in their ability to enhance the growth of the host. Similar results have been reported for the flatworm/alga association (Taylor, 1971b; Provasoli *et al.,* 1968). The importance of these differences in the most widespread and important symbiosis—between zooxanthellae and their invertebrate hosts—has far-reaching implications.

Comparison between the polyps infected with zooxanthellae from *Aiptasia* and those infected with algae from *Cassiopea* suggest some interesting possibilities. It appears that polyps infected with homologous algae achieve a somewhat larger biomass but produce fewer offspring than polyps infected with zooxanthellae from *Cassiopea.* Comparisons between final tentacle numbers in these two experimental infections is more complex. While polyps infected with algae from *Aiptasia* produce only slightly higher tentacle numbers than those infected with algae from *Cassiopea,* in both series the T_{max} values resulting from fitting the measurement data to the von Bertalanffy growth equation are markedly different. In almost all the experiments the T_{max} values are slightly larger than the observed final tentacle number. The only exceptions are the experiments with algae from *Cassiopea.* In the first series the T_{max} value is slightly below the final tentacle count while in the second series the T_{max} value is very much larger. In addition, in this series, the rate value, r, is smaller than in any of the other experiments. The explanation for this is probably that the polyps in the second series had not stopped growing when the experiment was terminated and hence their growth is not adequately described by the model. Inspection of Figure 1b supports this suggestion. These differences between the two "successful" algal strains raises questions about the precise way that zooxanthellae influence their hosts and the possibility that different strains may have qualitative as well as quantitative differences.

Perhaps the most fundamental question is whether the relative success of the different strains is related to a physiological or biochemical relationship between a particular host species and its normal zooxanthellar strain, or whether success is independent of the taxonomic position of the host but instead is a function of the ecological conditions in which the association is found. Schoenberg's (1976) evidence suggests that the answer may encompass both possibilities. Certainly his results do not support a one-to-one host/strain specificity. In fact, of the seven host species where he sampled more than a single individual, four host species had the same algae strain among individuals sampled, as determined by electrophoresis, while three of the hosts had different algal strains in different individuals. The mollusk-coelenterate differences shown in this paper, however, are suggestive of at least some phylogenetic specificity. Schoenberg's data do not suggest any clearly discernible geographical patterns in the distribution of strains of zooxanthellae among hosts.

Since neither simple host specificity nor geographic factors resolve the distributional patterns, a third possibility is that of ecological parameters. To date, no studies have been published on the photosynthetic requirements of different strains of zooxanthellae *in vitro.* Dustan's (1975) data however, suggest that

in vivo differences might exist between associations at different depths with differing light quantity and spectral quality. The necessity of the host's securing the algal symbiont ecologically suited for the site of settling has been suggested as a reason for maintaining open life cycles, where the host must become infected anew each generation (Kinzie, 1974).

It would not be unexpected if the final answer was in fact a combination of phylogenetic, geographical and ecological effects, but at least the way is now clear for a thorough investigation of this association which is such a dominant feature of tropical reef systems and a paradigm of symbiosis.

This research was partially supported by NIH Grants RR 08 125 and RR 07 026. T. Smith and J. Stimson assisted with statistical matters and W. Cooke, J. Stimson and P. Jokiel improved the manuscript by their suggestions and comments.

Summary

1. A method is given enabling the differential effects of different strains of zooxanthellae on host growth to be assessed. This technique uses the increase in the number of tentacles as the measure of growth.

2. Aposymbiotic polyps of the anemone *Aiptasia pulchella* reinfected with strains of *Symbiodinium microadriaticum* isolated from the anemone *Aiptasia pulchella* and the scyphozoan *Cassiopea xamachana* grow as well as normal *Aiptasia* polyps.

3. Aposymbiotic *Aiptasia* polyps reinfected with zooxanthellae from the gastropod *Melibe pilosa* and the clam *Tridacna maxima* grew no better than polyps lacking zooxanthellae.

4. These results lead to the conclusion that strains of zooxanthellae differ in their ability to enhance growth of *Aiptasia* polyps under the experimental conditions and that these differences may have important ecological consequences.

LITERATURE CITED

Clausen, C. D., and A. A. Roth, 1975. Estimation of coral growth rates from laboratory 45 Ca incorporation rates. *Mar. Biol.*, **33**: 85–91.

D'Elia, C. F., 1977. The uptake and release of dissolved phosphorous by reef corals. *Limnol. Oceanogr.*, **22**: 301–315.

D'Elia, C. F., and K. Webb, 1977. The dissolved nitrogen flux of reef corals. *Proc. Third Intl. Coral Reef Symp.*, **1**: 325–330.

Droop, M. P., 1963. Algae and invertebrates in symbiosis. Pages 171–199 in P. S. Nutman and B. Mosse, Eds., *Symbiotic Associations*. Cambridge University Press, New York, N. Y.

Dustan, P., 1975. Genecological differentiation in the reef-building coral *Montastrea annularis*. *Ph.D. Thesis, SUNY at Stony Brook*, N. Y., 300 pp.

Franzisket, L., 1969. The ratio of photosynthesis to respiration of reef-building corals during a 24-hour period. *Forma Functio*, **1**: 153–158.

Franzisket, L., 1970. The atrophy of hermatypic reef corals maintained in darkness and their subsequent regeneration in light. *Int. Rev. Gesamten. Hydrobiol.*, **55**: 1–12.

Freudenthal, H. P., 1962. *Symbiodinium* gen. nov. and *S. microadriaticum* sp. nov. a zooxanthella: taxonomy, life cycle, morphology. *J. Protozool.*, **9**: 45–52.

GOREAU, T. F., 1961. On the relation of calcification to primary productivity in reef building organisms. Pages 269–285 in H. M. Lenhoft and W. F. Loomis; Eds., *The Biology of Hydra.* University of Miami Press, Coral Gables, Fla.

JOHANNES, R. E., 1974. Sources of nutritional energy for reef corals. *Proc. Second Intl. Coral Reef Symp.*, **1**: 133–137.

JOKIEL, P. L., AND S. L. COLES, 1977. Effects of temperature on the mortality and growth of Hawaiian reef corals. *Mar. Biol.*, **43**: 201–208.

KARAKASHIAN, S. J., 1963. Growth of *Paramecium bursaria* as influenced by the presence of algal symbionts. *Physiol. Zool.*, **36**: 52–68.

KAWAGUTI, S., 1944. On the physiology of reef corals VII: Zooxanthellae of the reef corals is *Gymnodium* sp. dinoflagellata; its culture *in vitro*. *Palao Tropical Biological Station Studies*, **2**: 675–680.

KEVIN, M. J., W. T. HALL, J. J. A. MCLAUGHLIN, AND A. ZAHL, 1969. *Symbiodinium microadriaticum* Freudenthal, a revised taxonomic description, ultrastructure. *J. Phycol.*, **5**: 341–350.

KINZIE III, R. A., 1970. The ecology of the gorgonians (Cnidaria, Octocorallia) of Discovery Bay, Jamaica. *Ph.D. Thesis, Yale University,* New Haven, Ct., 107 pp.

KINZIE III, R. A., 1974. Experimental infection of aposymbiotic gorgonian polyps with zooxanthellae. *J. Exp. Mar. Biol. Ecol.*, **15**: 335–345.

KINZIE III, R. A., G. ARAKI, AND L. KIA, 1977. The specificity of the intracellular symbiosis of zooxanthellae from a range of invertebrate hosts. *Am. Zool.*, **17**: 908.

MCLAUGHLIN, J. J. A., AND P. A. ZAHL, 1959. Axenic zooxanthellae from various invertebrate hosts. *Ann. N. Y. Acad. Sci.*, **77**: 55–72.

MCLAUGHLIN, J. J. A., AND P. A. ZAHL, 1966. Endozoic algae. Pages 257–297 in S. M. Henry, Ed., *Symbiosis.* Academic Press, N. Y., London.

MUSCATINE, L., 1961. Symbiosis in marine and freshwater coelenterates. Pages 255–268 in H. M. Lenhoff and W. F. Loomis, Eds., *The Biology of Hydra.* University of Miami Press, Coral Gables, Florida.

MUSCATINE, L., 1971. Endosymbiosis of algae and coelenterates. Pages 179–191 in H. M. Lenhoff, L. Muscatine and L. V. Davis, Eds., *Experimental Coelenterate Biology.* University of Hawaii Press, Honolulu.

MUSCATINE, L., 1973. Nutrition of corals. Pages 77–115 in O. A. Jones and R. Endean, Eds., *Biology and geology of coral reefs,* Vol. 2, *Biology 1.* Academic Press, New York.

MUSCATINE, L., AND C. F. D'ELIA, 1978. The uptake, retention, and release of ammonium by reef corals. *Limnol. Oceanogr.*, **23**: 725–734.

MUSCATINE, L., AND H. M. LENHOFF, 1965. Symbiosis of hydra and algae II. Effects of limited food and starvation on growth of symbiotic and aposymbiotic hydra. *Biol. Bull.*, **129**: 316–328.

MUSCATINE, L. R., R. POOL, AND R. K. TRENCH, 1975. Symbiosis of algae and invertebrates: Aspects of the symbiont surface and the host symbiont interface. *Trans. Am. Microsc. Soc.*, **94**: 450–469.

MUSCATINE, L., AND J. W. PORTER, 1977. Reef Corals: Mutualistic symbioses adapted to nutrient-poor environments. *BioScience,* **27**: 454–460.

PORTER, J. W., 1976. Autotrophy, heterotrophy, and resource partitioning in Caribbean reef-building corals. *Am. Nat.*, **110**: 731–742.

PROVASOLI, L., 1966. Media and prospects for the cultivation of marine algae. Pages 63–75 in A. Watanabe and A. Hattori, Eds., *Cultures and collections of algae.* Proc. U. S. Japan Conf., Hakone Sept. 1966, *Jpn. Soc. Plant. Physiol.*

PROVASOLI, L., T. YAMASU, AND I. MANTON, 1968. Experiments on the resynthesis of symbiosis in *Convoluta* with different flagellate cultures. *J. Mar. Biol. Assoc. U.K.*, **48**: 469–579.

SCHOENBERG, D., 1976. Genetic variation and host specificity in the zooxanthella, *Symbiodinium microadriaticum* Freudenthal, an algal symbiont of marine invertebrates. *Ph.D. Thesis, Yale University,* New Haven, Ct., 183 pp.

SCHOENBERG, D. A., AND R. K. TRENCH, 1976. Specificity of symbioses between marine cnidarians and zooxanthellae. Pages 423–432 in G. O. Macke, Ed., *Coelenterate Ecology and Behavior.* Plenum Publishing, N. Y., N. Y.

STEELE, R. D., 1975. Stages in the life history of a symbiotic zooxanthellae in pellets extruded by its host *Aiptasia tagetes* (Duch. and Mich.) (Coelenterata, Anthozoa). *Biol. Bull.*, **149**: 590–600.

SUGIURA, Y., 1964. On the life history of rhizostome medusae II. Indispensability of zooxanthellae for strobilation in *Mastigias papua*. *Embryologica*, **8**: 223–233.

TAYLOR, D. L., 1971a. Ultrastructure of the 'zooxanthella' *Endodinium chattonii* in situ. *J. Mar. Biol. Assoc. U.K.*, **51**: 227–234.

TAYLOR, D. L., 1971b. On the symbiosis between *Amphidinium klebsii* (Dinophyceae) and *Amphiscolops langerhansi* (Turbellaria: Acoela) *J. Mar. Biol. Assoc. U.K.*, **51**: 301–313.

TAYLOR, D. L., 1973a. Algal symbionts of invertebrates. *Ann. Rev. Microbiol.*, **27**: 171–187.

TAYLOR, D. L., 1973b. The cellular interactions of algal-invertebrate symbiosis. *Adv. Mar. Biol.*, **11**: 1–56.

TAYLOR, D. L., 1974. Symbiotic marine algae: taxonomy and biological fitness. Pages 245–262 in W. Vernberg, Ed., *Symbiosis in the Sea*. University of South Carolina Press, Columbia, S. C.

TAYLOR, D. L., 1975. Symbiotic dinoflagellates. *Symp. Soc. Exp. Biol.*, **26**: 267–277.

TRENCH, R. K., 1971a. The physiology and biochemistry of zooxanthellae symbiotic with marine coelenterates. I. The assimilation of photosynthetic products of zooxanthellae by two marine coelenterates. *Proc. R. Soc. Lond. B. Biol. Sci.*, **177**: 225–235.

TRENCH, R. K., 1971b. The physiology and biochemistry of zooxanthellae symbiotic with marine coelenterates. II. Liberation of fixed ^{14}C by zooxanthellae *in vitro*. *Proc. R. Soc. Lond. B. Biol. Sci.*, **177**: 237–250.

TRENCH, R. K., 1971c. The physiology and biochemistry of zooxanthellae symbiotic with marine coelenterates. III. The effect of homogenates of host tissues on the excretion of photosynthetic products *in vitro* by zooxanthellae from two marine coelenterates. *Proc. R. Soc. Lond. B. Biol. Sci.*, **177**: 251–264.

TRENCH, R. K., 1974. Nutritional potentials in *Zoanthus sociatus* (Coelenterata, Anthozoa). *Helgol. Wiss. Meersunters*, **26**: 174–216.

VANDERMUELEN, J. H., AND L. MUSCATINE, 1974. Influence of symbiotic algae on calcification in reef corals: critique and progress report. Pages 1–19 in W. B. Vernberg, Ed., *Symbiosis in the Sea*. University of South Carolina Press, Columbia, S. C.

VON BERTALANFFY, L., 1938. A quantitative theory of organic growth. *Hum. Biol.*, **10**: 181–213.